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(54) **Method to extract and purify human genomic DNA.**

(57) Method to extract and purify human genomic DNA, which provides for the following steps:

- charging the sample, which undergoes a lysis treatment
- extracting with chloroform or other organic solvents
- centrifuging to eliminate the protein portion
- diluting in water and precipitating with cationic detergent
- filtering and adhering of the genomic DNA to a suitable filter
- washing the filter and eluting the human genomic DNA which is re-suspended in water.

EP 0 442 026 A2

METHOD TO EXTRACT AND PURIFY HUMAN GENOMIC DNA

This invention concerns a method to extract and purify genomic DNA from human total blood, tissues, sperm, cultured cells. The same method can be used also for extracting DNA from animal blood and tissues, virus (es. hepatitis), vegetal cells.

To be more exact, the invention provides for a method which can be partly reproduced manually and partly automatically in limited performance times.

The traditional method employed, for instance, by the "DNA Extractor" apparatus of the Applied Biosystem is known but is not satisfactory owing to its low percentage yield and its slowness; moreover, it employs as a reagent phenol which is expensive, irritant and neurotoxic. The present applicant has therefore tackled the problem of providing a method which enables higher percentage yields to be achieved more speedly and simply.

The method to extract and purify human genomic DNA is set forth and characterized in the main claim, while the dependent claims describe variants of the idea of the main solution.

The method according to the invention makes it possible to obtain in about twenty minutes what can be obtained otherwise in about four hours with a lower yield.

Moreover the method according to the invention can be automated at least partly with a suitable apparatus which provides the human genomic DNA in 10 to 15 minutes in a test-tube containing a small quantity of water (500-1000 microlitres for instance).

The method, according to the invention, for the extraction and purification of DNA, starting from human blood as a whole, provides for at least the following steps:

- charging the sample, which undergoes a lysis treatment
- extracting the protein with an organic solvent, chloroform for instance
- centrifuging to eliminate the protein portion
- diluting in water and precipiting with cationic detergent
- filtering and adherence of the genomic DNA to a suitable filter
- washing the filter and eluting the genomic DNA, which is resuspended in water.

The DNA obtained in this way can be employed in futher analysis:

1. Enzimatic restriction-Southern blot.
2. Amplification of specific genic fragments with the polymerase chain reaction (PCR).

Percentage yields as compared to the traditional methods vary between 120% and 150%, with a recovery of 40 micrograms per millilitre of blood.

To be more exact, the method according to the invention includes the following main steps:

STEP 1: charging a sample of blood, normally between 300 and 2500 microlitres but also a different amount, which undergoes a treatment of lysis by a cationic detergent for example tetradecyltrimethilammoniumbromide (TTAB) or dodecyltrimethilammoniumbromide (DTAB) both with sodium cloride at a concentration higher than 0.5 M.
This solution is mixed and heated up to 68 °C and incubated for a while (es. five minutes).

STEP 2: an extraction is made by adding 1 volume of chloroform or other organic solvents.

STEP 3: the mixture undergoes centrifuging with a normal bench centrifuge for some minutes to eliminate the protein portion which forms a clog with the organic phase.

STEP 4: after centrifuging, to the acqueos phase are added a quantity of water to decrease the ionic strenght below 0,5 M NaCl, and a cationic detergent (for instance a solution of 5% of Cetyl-trimetil-ammonium bromide), so that precipitation of the cationic detergent micellar complex-DNA takes place after a short mixing operation.

STEP 5: the solution thus obtained and containing the micellar-DNA complex then undergoes filtration. The ultrafiltration takes place with a filter (for instance, sintered borosilicate glass or an organic matrix like polypropilene or polyethylene) of known and tested porosity (pore size between 5 and 15 micron), which retains the DNA-cationic detergent micellar complex in a satisfactory way. The hydrophilic surface enables DNA to be recovered easily and speedly after the washing operations. The organic matrix filter allows a slower recovery of DNA so it is not commonly used at the moment.
As a genomic DNA is immobilized on the filter, it is then eluted.

STEP 6: this elution takes place after a series of washing of the filter:

- a first wash is carried out in an acqueos solution of a low ionic strenght to eliminate the excess of detergent;
- a second wash is carried out with a mixtures of alcohol and an ionic solution, for instance of the type NaCl 0,2 M/ 70% ethanol, to change the DNA-cationic detergent complex into DNA-Na;
- a third wash is carried out with a mixture of alcohol and a solution of low ionic

strenght to remove the excess of salts, for instance NaCl type (for instance, 70% ethanol and 30% water).

After having removed every trace of alcohol from the filter by a current of air, the DNA is recovered by washing the filter with an acqueos solution of a low ionic strenght (water, for instance) at room temperature or by heating, for instance at 68° C.

Claims

1. Method to extract genomic DNA from blood and also tissues and cultured cells, which provides for the following steps:
   - charging the sample, which undergoes a lysis treatment
   - extracting the sample
   - centrifuging to eliminate the protein portion
   - diluting in water and precipitating with cationic detergent
   - filtering and adherence of the genomic DNA to a suitable filter, which is resuspended in water.

2. Method as claimed in claim 1, in which the lysis solution contains cationic detergent with a hydrocarbonic chain of size comprised between C8 and C18, for instance the C14 tetradecil-trimethyl-ammonium bromide, or C12 dodecyltrimethyl-ammonium bromide and sodium chloride (more than 0,5 M).

3. Method as claimed in any claim 1 or 2, in which a complex containing DNA undergoes ultrafiltration on a porous (borosilicate) glass filter or another filter material.

4. Method as claimed in any claim hereinbefore, in which total blood, tissues, or cultured cells are charged into a tube.

5. Method as claimed in any claim hereinbefore, in which the washings of the filter comprise at least:
   - a first wash in an acqueos solution of a low ionic strenght;
   - a second wash with a mixture of alcohol and ionic solution;
   - a third wash with alcohol and a solution of low ionic strenght.

6. Method as claimed in any claim hereinbefore, in which a small quantity of water is used.

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EUROPEAN SEARCH REPORT

Application Number

EP 90 31 3173

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
A	EP-A-240191 (SEIKO INSTRUMENTS INC.) * column 2, line 35 - column 3, line 49 *	1-4	C12N15/10 C12P19/34
A	WO-A-8901035 (EUROPÄISCHES LABORATORIUM FUR MOLEKULARBIOLOGIE_(EMBL)) * page 3, line 12 - page 4, line 30 *	1	
P,A	EP-A-376080 (TALENT SRL) * column 2, line 35 - column 4, line 29 *	1-4	

TECHNICAL FIELDS SEARCHED (Int. Cl.5)

C12N
C12P

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	23 FEBRUARY 1991	ANDRES S.M.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

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